Rapid Report

In vivo pH and metabolite changes during a single contraction in rat uterine smooth muscle

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1. We have used ^{31}P NMR spectroscopy to measure metabolites and pH_i at three periods during a phasic contraction of the uterus, *in vivo*, to determine whether they change as a consequence of contraction. The regular uterine contractions were recorded via a balloon catheter in the uterine lumen. Each phasic contraction was divided into three parts: the period between contractions (rest), the development of force (up) and the relaxation of force (down). The NMR data were summed separately from each of these three periods over 20–40 successive contractions.

2. Significant changes in ATP, phosphocreatine (PCr) and inorganic phosphate (P_i) occurred during the contraction. [ATP] fell from 2·0 to 1·6 mM and [PCr] from 2·6 to 2·0 mM during the up period, while [P_i] increased from 2·2 to 2·8 mM. Recovery of ATP and PCr occurred during the relaxation part of the contraction, whereas P_i did not fully recover until the contraction was complete.

3. Significant acidification from pH 7·28 ± 0·02 at rest to 7·16 ± 0·02, occurred with contraction. This acidification is greater than that previously reported for *in vitro* uterine preparations. Measurements of uterine blood flow show that it decreased with contraction. Therefore, ischaemia, in addition to the metabolic consequences of contraction, may account for the larger acidification observed *in vivo*.

4. Lowering pH_i in an *in vitro* uterine preparation by a similar level to that found *in vivo* produced a significant reduction of the phasic contractions. Thus we propose that these changes, especially the fall in pH_i during force development, feed back negatively on the contraction to limit its strength, and may help prevent uterine ischaemia and fetal hypoxia during labour.

Phasic contractions of smooth muscle are necessary for many vital functions, e.g. childbirth and digestion. It is therefore important that the metabolic demands of contraction can be met and that contractile activity can be regulated. Smooth muscle has relatively little phosphocreatine (PCr) (around 2–4 mM) and ATP is present at around 3–5 mM (Wray, 1990). No data are available concerning the metabolic consequences of contractile activity of smooth muscle *in vivo*. From *in vitro* work, mainly on tonically activated vascular preparations, it has been calculated that ATP production only rises 2- to 3-fold with contraction, compared to over 30-fold in skeletal muscle (Paul, 1980). The rate of ATP utilization has been estimated to increase 2- to 4-fold with contraction in portal vein (Paul, 1980). Many of these calculations are, however, indirect and it is not clear therefore what will happen to [ATP] during phasic activity of smooth muscle.

In vivo the magnitude of any changes in metabolites will depend not only on contractile activity but also on blood supply to the tissue (Taggart & Wray, 1998). It is known that, in the uteri of large animals and women, blood flow is reduced during contraction, as a consequence of the active tissue compressing the blood vessels supplying the uterus (Greiss, 1965). Clearly any such reductions in blood supply will limit oxygen and glucose to the active tissue, and thereby affect ATP production. There are few data examining the relationship between blood flow and metabolites in smooth muscle, but we have recently shown in rats that there is a linear relationship between blood flow and [ATP] and [PCr] (Larcombe-McDouall *et al.* 1998*b*). Contractile activity also falls as blood flow is reduced; even small reductions of flow were functionally significant (Larcombe-McDouall *et al.* 1998*b*). Thus considering both the metabolic demands of contraction, and the reduction of blood flow

which occurs during uterine contraction, we addressed the question, 'Do ATP and other related metabolites, change during the course of a single contraction?'

Changes of intracellular pH (pH_i) with contractile activity may be anticipated due to increased lactate production, ATP hydrolysis and PCr breakdown (Taggart & Wray, 1995). *In vitro* studies of phasic smooth muscle have shown that contraction is associated with a small transient acidification (Taggart & Wray, 1993; Naderali *et al.* 1997). In addition to the above metabolic causes, these acidifications have been related to the counter-transport of protons into the cell, when Ca^{2+} is extruded on the surface membrane Ca^{2+}-ATPase (Naderali *et al.* 1997). It is not known whether such pH transients occur *in vivo*, during uterine contractions.

The above discussion was focused on whether metabolites and pH_i alter as a consequence of contractile activity. However, it must also be considered that, if any such changes do occur, they may, in turn, influence contraction. It has been clearly shown, for example, that spontaneous uterine contractions in pregnant and non-pregnant myometrium are decreased with acidification (Wray *et al.* 1992; Taggart & Wray, 1993). Similarly, if the free energy of ATP hydrolysis decreases, this could also influence contractile ability. Thus the question of whether changes in pH_i or [ATP] associated with contraction do indeed depress contraction needs to be addressed.

The primary purpose of this study was to determine whether metabolite and pH_i changes occur during the course of a phasic contraction, *in vivo*. We have used NMR spectroscopy, *in vivo*, to determine metabolite concentrations and pH_i in the uterus and find significant changes during a uterine contraction. These are the first *in vivo* data showing such changes in any smooth muscle. We also performed *in vitro* experiments to show that the changes may be functionally significant. We propose that these changes provide a negative feedback mechanism to limit the contraction. Some of these data were presented to The Physiological Society (Larcombe-McDouall *et al.* 1998*a*).

METHODS

Animals

Day 1 post-partum uterus was studied as it produces regular and reproducible contractions and provides sufficient tissue for the NMR spectra. In addition, the post-partum uterus produces particularly regular contractions. Pregnant uterus would be interesting to study, but the response of fetuses complicates interpretation of the data. Non-pregnant uteri are too small to acquire sufficient data for this study in a reasonable period of time. In addition, changes in metabolites with oestrus could complicate the data. Sprague–Dawley rats were anaesthetized with urethane (I.P. 1 ml (200 g body weight)$^{-1}$ of a 36% (w/v) solution). This is a long-lasting anaesthetic which was sufficient for surgery and obtaining the NMR data. A small mid-portion of uterine horn was exteriorized, and a balloon catheter inserted into the uterus, to provide measurements of intrauterine pressure. The integral of the contraction data was calculated, to take into account variations of frequency and amplitude of uterine force. A 1 cm diameter, 3-turn NMR coil was placed on top of the mid-uterine portion containing the recording balloon. In this way metabolic and contractile activity were obtained from the same region of tissue (see Harrison *et al.* 1994, for further details). The overwhelming (> 90%) cellular component of the uterus arises from smooth muscle cells in the myometrium, and therefore the data obtained are taken as arising from these cells (Wynn, 1977). The uterus was covered with cling film to keep it warm and moist and the anaesthetized rat was placed on a heated cradle in the NMR spectrometer. The rat's core temperature was maintained at 37 ± 1 °C. At the end of the experiment the animal was killed, while still anaesthetized, by cervical dislocation. In some experiments, detailed in the text, the piece of uterus which had been used for the *in vivo* experiments was excised and then used for the *in vitro* pH_i measurements (see below).

NMR spectroscopy

^{31}P spectra were obtained at 81·1 MHz using a 4·7 T, 15 cm bore Biospec 1 NMR spectrometer. Radiofrequency (RF) pulses of 4 μs duration (45 deg) were repeated every 1·4 s. As shown in Fig. 1, the contractions were divided into three parts, and the NMR data were obtained selectively from these three parts over 20–40 successive contractions. Between 10 and 20 RF pulses were applied to each part of the contraction. The metabolic and pH_i data between the three parts of the contraction cycle were then compared. (Five animals exhibiting irregular activity were not used in this study.) Data points were zero filled from the acquired points to 4096, and line broadening of 20 Hz was used before Fourier transforming the data. Changes in metabolite concentrations were obtained through deconvolution and integration using the WIN-NMR 1D program (Bruker Spectrospin). The area of the spectrum around P_i is particularly complex and even with deconvolution the absolute value of the P_i peak may be over-estimated and thus percentage changes in concentration during contraction and relaxation may be underestimated. By setting the mean value over the entire contraction cycle of the metabolite concentrations to that of previously published values of metabolite concentrations in the post-partum rat (Dawson & Wray, 1985), we could calculate the absolute changes in those metabolites during the cycle. Intracellular pH was calculated from the resonance position of P_i, with respect to PCr (which was set to 0 parts per million, p.p.m.), using the following modification of the Henderson–Hasselbalch equation:

$$\mathrm{pH} = \mathrm{pK} + \log_{10} \frac{\delta - \delta_1}{\delta_2 - \delta},$$

where δ is the observed P_i position, and δ_1 and δ_2 are the chemical shifts of $H_2PO_4^-$ and HPO_4^{2-} (3·28 and 5·69), respectively. The pK used was 6·73.

In vitro pH_i measurements

Following killing of the rat by cervical dislocation under either urethane (as above) or chloroform anaesthesia, strips of longitudinal myometrium were dissected and loaded with the membrane-permeant form of the pH-sensitive indicator carboxy-SNARF (5 μM), at room temperature for 1–4 h. This loading has previously been shown not to perturb tissue function or buffering (Taggart & Wray, 1993; Bullock *et al.* 1998). The tissues were transferred to a 200 μl bath on the stage of an inverted microscope, and one end was attached to a tension transducer. The tissues were superfused with oxygenated solution (36 ± 1 °C) of the following composition (mM): NaCl, 154; KCl, 5·4; $MgSO_4$, 1·2; glucose, 1·2; and $CaCl_2$, 2·5; and buffered with 11 mM Hepes. Intracellular acidification, at constant external pH (7·4), was obtained by isosmotically substituting up to 40 mM sodium butyrate for NaCl in the

perfusate. The tissues were excited at 530 nm and the emission signals at 590 and 640 nm recorded. The fluorescence ratio, F_{590}/F_{640}, of these two signals was used to measure pH_i. The pH_i records were calibrated with a mock intracellular solution, as previously described (Taggart & Wray, 1993).

Laser Doppler flow measurements

In some experiments blood flow to the uterus was recorded using a Laser Doppler flowmeter (Moore Instruments). A 1 mm^2 probe was placed upon the portion of uterus being studied and relative flux (flow) recorded. At the end of the experiments, when the animal was dead, the flowmeter value was recorded and this value taken as the baseline (0%) value. In some experiments blood flow to the uterus was reversibly occluded using inflatable balloons positioned next to the artery, as detailed in Larcombe-McDouall *et al.* (1998*b*).

Statistics

Figures given are mean values ± S.E.M. and n is the number of animals. Differences were taken as significant where P values were $< 0{\cdot}05$ in either ANOVA or the appropriate Student's t test.

RESULTS

Metabolite and pH_i changes with contraction

Figure 1*A* shows a typical *in vivo* recording of uterine contractions and the three sections the contractions were divided into for NMR data acquisition. Figure 1*B* illustrates the ^{31}P NMR spectrum from the uterus, and shows the typical peaks from ATP, PCr and P_i. It also shows the NMR spectra from the period over which the uterus was developing force (up) and decreasing force (down), and during the period between contractions (rest). It can be seen that $[P_i]$ was elevated and [ATP] was decreased in the up spectrum relative to the rest spectrum. An acidic shift in pH_i can also be seen, i.e. a rightward shift in the P_i resonance position. Mean data from 17 animals are shown in Fig. 2. Contraction produced significant reductions in [ATP] and [PCr] and a significant increase in $[P_i]$ compared with resting levels. The mean resting value of pH_i was $7{\cdot}28 \pm 0{\cdot}02$, falling to $7{\cdot}16 \pm 0{\cdot}02$ with contraction ($n = 17$). Recovery of ATP and PCr occurred during the relaxation part of the contraction (down), such that their values were not significantly different from those occurring at rest. The $[P_i]$ fell during this period, but recovery was incomplete until the contraction was over. Recovery of pH_i was also incomplete until the rest period, and thus during the downstroke it was still significantly more acid than at rest. The calculated changes occurring in metabolites during the contraction showed decreases in [ATP] from 2·0 to 1·6 mM and [PCr] from 2·6 to 2·0 mM, and an increase in $[P_i]$ from 2·2 to 2·8 mM.

The above data show significant changes in metabolites and pH_i with contraction. In addressing the question of what causes these changes, one putative mechanism we considered was changes in blood flow. Earlier data in sheep and women (Greiss, 1965; Brar *et al.* 1988) had shown falls in uterine blood flow with contraction. We therefore firstly wanted to determine: (i) whether such changes occur in the post-partum rat uterus and (ii) whether such changes can produce metabolite changes.

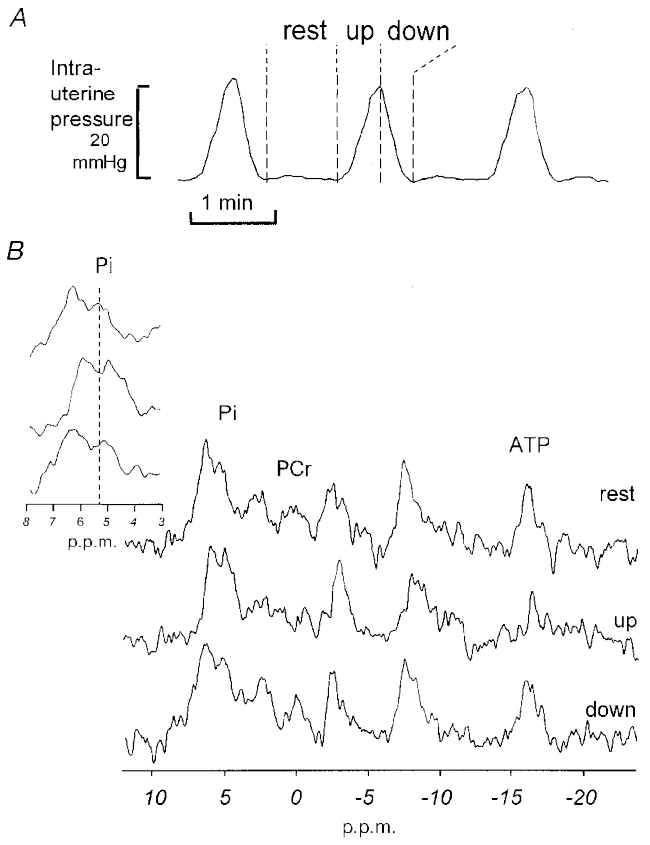

Figure 1. Uterine contraction and associated NMR spectra

A, recording of *in vivo* uterine contractions showing how each contraction cycle was divided into three parts: rest, upstroke (up) and downstroke (down). *B*, ^{31}P NMR spectra from the uterus *in vivo*, obtained via a small surface coil put over the area of the uterus containing the pressure-recording balloon. The spectra were obtained during the three parts of the contraction described in *A*. Peaks from ATP, PCr and P_i can be seen. The inset shows the spectral region around P_i, to show the acidic shift in pH, i.e. rightward shift, which occurred with contraction. p.p.m., parts per million.

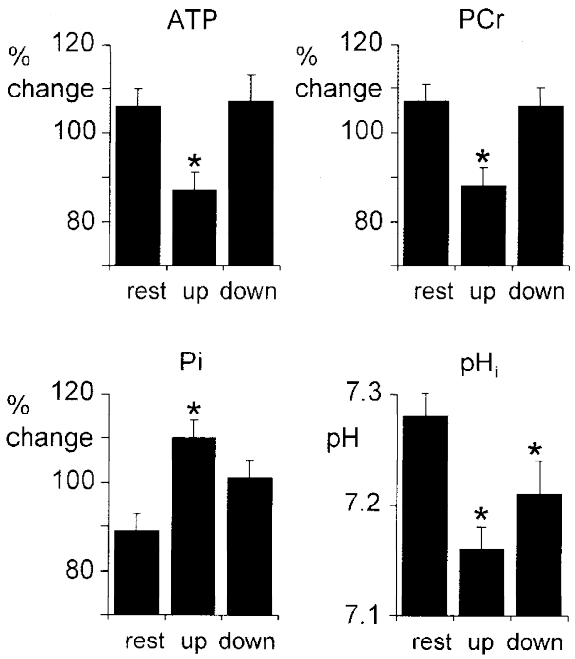

Figure 2. *In vivo* changes in the concentrations of ATP, PCr and P_i, and in pH_i during a uterine contraction

Mean values ± S.E.M. from 17 animals. The metabolite values are expressed relative to those found over the entire contraction cycle (100%), via ^{31}P NMR spectroscopy. * Significantly different from the value at rest.

The effect of blood flow on metabolites

Figure 3 shows a simultaneous recording of uterine blood flow and contractions. It can be seen that the contractions were associated with reductions of uterine blood flow. In 15 preparations the reductions ranged from 15 to 58%, the mean reduction being 33 ± 3%. To directly assess whether such a reduction of blood flow could produce changes in uterine metabolites and pH_i, we occluded the uterine artery to produce similar reductions in flow. NMR spectra were acquired during the 10–20 min occlusion period. The mean effects on metabolites and pH_i of such occlusions ($n = 9$; mean, 33 ± 4%; range, 20–50%; where 100% is complete occlusion) are shown in Fig. 3*B*. It can be clearly seen that, compared with control levels, [ATP], [PCr] and pH_i fell significantly and $[P_i]$ increased significantly as a result of this occlusion. Furthermore, the effects of occlusion on

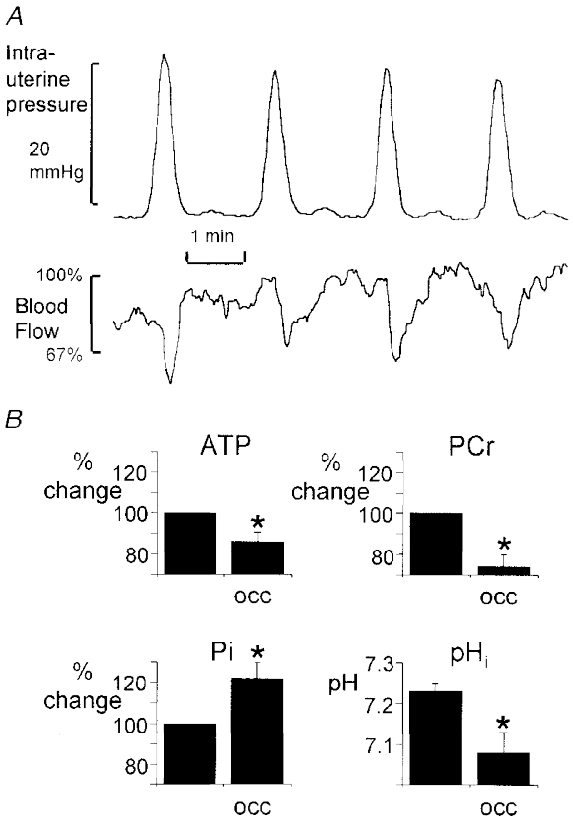

Figure 3. Changes of blood flow and metabolites in the uterus

A, simultaneous recording of uterine contractions and blood flow, measured via an intrauterine balloon and a laser Doppler flow probe on the uterine artery. The blood flow baseline (0%) was obtained at the end of the experiment, when the rat was dead. *B*, the mean effects on metabolites and pH_i of occlusion of the uterine artery over the range 20–50% (no occlusion = 100%, $n = 9$). * Significantly different from control value.

metabolites and pH_i were similar to those found over the contraction cycle (Fig. 2). It therefore appears likely that the metabolite and pH_i changes are secondary to occlusion. The next question we addressed was whether such changes in blood flow or metabolites would have any functional effect.

The effects of reducing blood flow on contractions

We have previously found that when blood flow to the uterus is reduced, force falls (Harrison *et al.* 1994; Larcombe-McDouall *et al.* 1998*b*). The aim of the present experiments was to reduce blood flow to a comparable level to that occurring during spontaneous contractions and confirm that force is also reduced at this level.

Figure 4*A* shows the effect, *in vivo*, of such a reduction in blood flow (mean reduction, $35 \pm 4\%$; $n = 9$). Contractions remained, but were reduced in amplitude by $13 \pm 6\%$ (100%, control). These data strongly suggest therefore that there will be an inhibitory effect on the contraction cycle as the blood flow is reduced. The data obtained during the contraction cycle (Fig. 2) demonstrated a significant fall in pH_i. Previous work on pregnant and non-pregnant rat uterus had shown that acidification reduced uterine contractions. If acidification had a similar effect on the post-partum uteri used in this study, then this might point to the mechanism whereby force is reduced when blood flow is reduced. Thus the next experiments addressed the question whether similar pH_i changes to those occurring *in vivo* with contraction reduce uterine contractile activity.

The effect of acidification on post-partum uterus

In three *in vitro* preparations simultaneous measurements of pH_i and force were made. Resting pH_i was $7{\cdot}11 \pm 0{\cdot}13$. Addition of 10 mM sodium butyrate produced a mean reduction of $0{\cdot}12 \pm 0{\cdot}02$ pH units, and a reduction in force in all three preparations, which was pH_i dependent; larger acidifications abolished contractions (not shown). To more directly relate the *in vitro* and *in vivo* data, in two preparations after *in vivo* data had been obtained, the same piece of tissue was excised, and the *in vitro* effects of reducing pH_i were then determined. Figure 4*B* shows that when pH_i was directly reduced *in vitro*, by a similar amount to that which occurred *in vivo*, contractions were reduced; these data were obtained from the same tissue as shown in Fig. 4*A*. Thus it can be seen that both occlusion and reducing pH_i reduce contractile activity in the uterus post-partum.

DISCUSSION

Using NMR spectroscopy *in vivo*, we have found significant changes in metabolites and pH_i during a single contraction. This is the first demonstration of such *in vivo* changes in any muscle. The changes produced were rapidly restored over the contraction cycle and were of the order of 10–20%. The mechanism underlying the changes is considered to involve the metabolic and ionic requirements of the contractile process, coupled to a reduction of blood flow consequent to the contraction. We consider that these changes and in particular the fall in pH_i will inhibit force production, i.e. the intrinsic changes in metabolites and pH produced by contraction feed back on the contractile process, and help limit it. Such a mechanism, along with time-dependent changes in electrical activity, will help control the phasic contractions and prevent harmfully low [ATP] and pH changes from occurring.

Phasic smooth muscle contractions of the uterus have the advantage of being slow (1–2 min) compared with those of some other smooth muscles, e.g. portal vein and the heart. This makes it easier to switch data acquisition from one part of the contraction to another, and also ensures that the NMR excitation pulse and acquisition times can be confined to the part of the cycle under study. The uterus is also not subject to motion during the contraction or due to respiration which would significantly alter its position relative to the NMR coil. This is confirmed by the opposite changes in metabolites relative to each other, e.g. P_i and ATP, rather than parallel changes which would occur due to any movement.

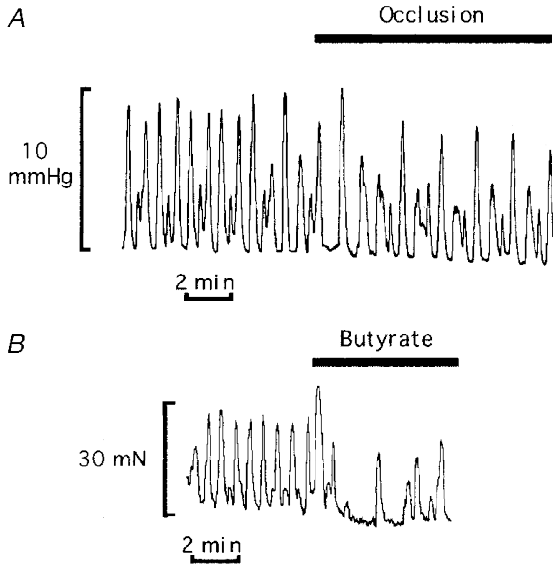

Figure 4. The effect on contractions of arterial occlusion and reduced pH_i

A, the effect of occlusion on uterine force production *in vivo*. *B*, using the same tissue studied in *A*, the effect of acidification on uterine force production *in vitro* was determined by adding 10 mM butyrate to the perfusate. This provided a mean change of pH_i of $0{\cdot}12 \pm 0{\cdot}02$ pH units ($n = 5$; mean resting pH_i, $7{\cdot}11 \pm 0{\cdot}13$) and was associated with a significant decrease in force. The effects of pH and occlusion were fully reversible (not shown). pH_i values were obtained using SNARF, as described in Taggart & Wray (1993).

The changes in metabolites and pH_i were relatively modest. Similar small changes have recently been reported during an *in vitro* (Illing *et al.* 1998) and an *in vivo* (Toyo-oka *et al.* 1986) study of cardiac muscle. Previously, in smooth muscle, *in vitro* studies have been made of metabolites during prolonged contractions, e.g. high K^+ depolarization (Vermue & Nicolay, 1983; Adams & Dillon, 1989) following metabolic inhibition (Hellstrand & Vogel, 1985) or substrate withdrawal (Hardin *et al.* 1992). The above reports varied from finding no changes to reporting small but significant changes, mostly due to impairment of oxidative phosphorylation. During contraction, increased ATP hydrolysis, compared with resting levels, would be required for cross-bridge cycling, phosphorylation of the myosin light chains and any additional ion pumping, e.g. due to elevated $[Ca^{2+}]$. Consistent with this are reports that ATP synthesis in smooth muscle is modestly increased (2- to 3-fold) between 'resting' and 'activated' contractions (Hellstrand, 1996) and ATP utilization rises 3- to 4-fold (Paul, 1980).

Clearly a decline in the free energy of ATP hydrolysis would be expected to affect contraction. From our data, and assuming an [ADP] of 25 μM at rest and 100 μM during contraction (Hardin *et al.* 1992; Dillon, 1996), and $\Delta G°$ ATP $= -32{\cdot}3$ kJ mol^{-1} at 38 °C and pH 7·2, the free energy of ATP hydrolysis can be calculated to change from $-58{\cdot}3$ to $-53{\cdot}7$ kJ mol^{-1}. This fall will influence contraction as well as other cellular functions but is not large enough to limit ATP hydrolysis. It is, however, also clear that the individual metabolites can affect force production in smooth muscle. Dillon (1996) suggests from experiments on hypoxic K^+-stimulated vascular smooth muscle, the following order of importance: $P_i >$ free energy $=$ pH $>$ PCr $>$ ATP $>$ ADP. In the uterus increasing $[P_i]$ to 6–11 mM in permeabilized preparations did depress Ca^{2+}-activated force (Crichton *et al.* 1993). However, the small change in $[P_i]$ found during spontaneous contractions (from 2·2 to 2·8 mM) would have little effect on uterine contraction. Similarly, the fall of [ATP], though significant, is not to below 1 mM, and therefore it is unlikely to affect the activity of ATP-fuelled ionic and enzymatic processes necessary for contraction (Carafoli, 1987; Barany & Barany, 1990). This simplistic analysis does not take into account local changes in [ATP], e.g. around the myofilaments, which might arise if diffusion was limited or metabolism was compartmentalized (Paul, 1980), as little data concerning this are available. The change of pH_i was found to be functionally significant, and will therefore be discussed next.

The effect of pH_i alteration

Although there is little *in vitro* work on uterine metabolites with which to compare our *in vivo* data, this is not the case for pH_i. Using SNARF to measure pH_i, small acidifications (~0·04 pH units) were found to accompany spontaneous contractions (Taggart & Wray, 1993). Increasing uterine contraction, by depolarization, produced larger acidifications. Recent work has shown the acidification to be linked to Ca^{2+} efflux on the plasma membrane Ca^{2+}-ATPase (Naderali *et al.* 1997). The findings of pH changes with contraction observed with a different methodology and preparation clearly reinforce the observations made *in vivo* in the current study. The pH_i changes recorded *in vivo* were, however, around 3 times larger than those observed *in vitro* despite similar contractile patterns and temperature. Thus while an effect on pH_i due to the elevated Ca^{2+} with contraction stimulating the Ca^{2+}-ATPase will be expected *in vivo*, the larger acidification suggests an additional mechanism or effect is present *in vivo*. Our data measuring blood flow during contraction suggest a putative mechanism, which is discussed below. Unlike the changes in metabolites, changes in pH_i have previously been demonstrated to be potent modulators of uterine force; acidification can inhibit spontaneous force generation (Taggart & Wray, 1993). That this was also the case for post-partum uterus was found in this study. Acidification caused by application of a weak acid significantly reduced force. In pregnant uterine tissue this effect of acidification has been shown to be due to a reduction in Ca^{2+} current (Shmigol *et al.* 1995) and hence intracellular $[Ca^{2+}]$ (Taggart *et al.* 1997). This suggests that of all the changes occurring due to contraction, pH_i is the one most likely to have a functional effect.

The relationship between blood flow, metabolites and contraction

An obvious difference between previous *in vitro* studies and the present study is that *in vivo* the uterus depends on its blood supply to maintain oxygenation and metabolites. As others have previously shown (Greiss, 1965; Brar *et al.* 1988) and we show here for the rat, uterine contractions produce significant reductions in blood flow to the uterus. The delay between contractions and blood flow change apparent in Fig. 3*A* is likely to be due to experimental reasons – the Doppler flow probe had to be positioned a small (~1 cm) distance from the NMR surface coil. Thus during the course of contraction *in vivo* the normal delivery and removal of metabolic fuels and waste products is curtailed. Clearly changes in metabolites and pH_i might be expected as a consequence of this, as the uterus switches from aerobic to anaerobic metabolism (Wray, 1990).

When uterine blood flow was reduced to values similar to those occurring during contraction, a small reduction in force occurred. This agrees with our previous data showing that uterine contractions depend on an adequate blood supply to the uterus, i.e. contractile activity decreases linearly with flow (Larcombe-McDouall *et al.* 1998*b*). Although not so apparent in Fig. 4*A*, contractions were affected within 1–2 min of producing occlusion on the artery, which is a small distance away from the uterus where contractions were measured. It may also be that prolonged occlusions produce additional changes to those seen during phasic contractions. These data are also supported by *in vitro* data simulating the effects of hypoxia on uterine contractions by inhibiting oxidative metabolism with cyanide – similar

changes in metabolites and pH_i occur and force is reduced (Wray, 1990; Taggart *et al.* 1997). This suggests that the metabolic demands of contraction, coupled with the reduction in blood flow, are sufficient to account for the changes seen.

Feedback of metabolites and pH_i on contraction

The data in this study lead to the following proposal: changes in pH_i along with metabolites during the contractile cycle are likely to be functionally significant and reduce force. This would form a feedback mechanism:

Force development → ↓blood flow + ↑metabolic demand →

↓ATP + ↓pH_i → inhibit force → ↓contraction →

restoration of blood flow, metabolites and pH_i →

Such a feedback mechanism would limit the forcefulness of contractions and prevent damagingly low levels of [ATP] or pH_i occurring, along with ischaemic damage to tissue. In the pregnant uterus it would also serve to limit the reduction in blood flow to the fetus. If the feedback mechanism fails, or is over-ridden, e.g. by oxytocin augmentation of labour, then the metabolite levels may decrease too much for the uterus to be capable of recovery and fetal hypoxia may occur. The fact that these experiments were performed *in vivo* means that the changes occur despite multiple contractile and metabolic drives on the uterus. Given the similar contractile mechanism present in other smooth muscle, it may be expected that the proposed feedback mechanism helps control contractile activity in other tissues.

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